SUPPORT FOR THE AMENDMENT

Claims 1-30 and 47-53 have been canceled herein. Such cancellation is without prejudice on the merits to further prosecution of these claims in one or more divisional applications. Claims 31 and 34 have been amended herein. Claims 31-46 remain in the application.

Claim 31 as amended enjoys verbatim support in the specification at page 16, lines 11-13 ("[T]he subject assay can be used to determine the presence and/or activity of any lipid of phospholipid kinase...."); and at page 18, lines 30-31 ("[T]he subject assay can be used to determine the presence and/or activity of any lipid or phospholipid phosphatase....").

Claim 34 has been amended solely to add the accidentally omitted word "substrate" after the word "enzyme."

No new matter is added.

REMARKS

The following remarks address the issues in the Office Action in the order in which they appear. Favorable reconsideration is respectfully requested.

Rejection of Claim 34 Under 35 USC §112, Second Paragraph:

This rejection is believed to have been obviated by appropriate amendment to the claim. Specifically, Claim 34 has been amended (in accordance with the Examiner's recommendation) to recite that it is the "enzyme substrate" that is bound to the matrix, and not the enzyme itself.

Applicants submit that this rejection has now been overcome. Withdrawal of the same is respectfully requested.

Rejection of Claim 31 Under 35 U.S.C. § 102(b) Over Toomik et al.:

This rejection is believed to have been overcome, in part, by appropriate amendment to the claim, and is, in part, respectfully traversed.

Applicants submit that the rejection of Claim 31 in view of Toomik et al. has been overcome in large part by amending the claim to require that the enzyme substrate that is fixed to the binding matrix be specifically reactive with a lipid kinase, a phospholipid kinase, a lipid phosphatase, and a phospholipid phosphatase. Insofar as the Toomik et al. paper fails entirely to address any of these types of enzyme substrates, Applicants submit that this rejection has been overcome.

Specifically, the Toomik et al. reference is limited entirely to a method that utilizes polypeptide substrates for protein kinase A, protein kinase C, casein kinases I and II, and acid phosphatase. Applicants respectfully note that all of these enzymes described in the Toomik et al. reference act on polypeptide substrates. In stark contrast, Claim 31 requires that the substrate used be specifically reactive with lipid and phospholipid kinases and phosphatases. These substrates, which are lipids and phospholipids, and which are positively required by the language of Claim 31, are neither disclosed nor suggested in the Toomik et al. reference. Therefore, the Toomik et al. reference does not anticipate Claim 31.

The rejection is also traversed, in part, because Claim 31 requires that an enzyme substrate be contacted with a binding matrix, such that the enzyme substrate "is fixed" to the matrix. This approach is fundamentally distinct from the approach taken by Toomik et al., which is limited solely to synthesizing an immobilized polypeptide substrate from scratch, residue-by-residue. Therefore, the approach described by Toomik et al. is necessarily limited to: 1) polypeptide substrates; and 2) polypeptide substrates that are amenable to solid-phase peptide synthesis. The Toomik et al. reference simply does not discuss or suggest in any fashion an assay for non-polypeptide substrates, nor does it address how to assay for a substrate that cannot be synthesized from scratch using solid-phase peptide synthesis.

In short, the Toomik et al. reference is absolutely silent regarding an enzyme assay for enzymes that act on lipid and phospholipid substrates. Insofar as Claim 31 specifically recites that the substrates must be specific for lipid and phosphlipid kinases

and phosphatases (and are therefore themselves lipids and phospholipids), this rejection is untenable.

Addressing this aspect of the Toomik et al. paper, the paper describes using a commercial kit sold under the trademark "SPOTs." The kits are made by Cambridge Research Biochemicals and distributed by Sigma/Genosys. For the Examiner's convenience, a copy of the SPOTs product literature is attached hereto as Exhibit A. The SPOTs-brand kit centers around a membrane divided into 96 discrete domains. Each domain contains reactive amino groups where solid-phase peptide synthesis can be initiated. Thus, when using the SPOTs-brand kit, each polypeptidic substrate is constructed, residue-by-residue, starting from the reactive amino groups present on the SPOTs membrane. See Toomik et al., page 7, left-hand column, last paragraph and extending to the middle column. The Toomik et al. reference, therefore, is literally limited to enzymes whose substrates are a polypeptides having "a strictly defined amino acid sequence" and which can be "synthesized on a separate spot using an activated cellulose membrane." *Id.*

Claim 31, however, requires that the "enzyme substrate," that is, the fully-formed enzyme substrate, be contacted with the matrix. This is distinct from Toomik et al.'s approach, which requires that the substrate be synthesized de novo. And, as noted above, the substrates recited in Claim 31 (lipids and phospholipids) are wholly distinct from the polypeptide substrates described by Toomik et al.

Applicants therefore submit that the rejection of Claim 31 under §102(b) in view of Toomik et al. is untenable. Withdrawal of the same is respectfully requested.

Rejection of Claims 32-43 Under 35 U.S.C. 103(a) Over Toomik et al. in View of Promega Technical Bulletin No. 547, and in Further View of Sartobind Product Literature:

This rejection is respectfully traversed because the Office has not presented a *prima facie* case of obviousness. In short, **the combination** neither teaches nor fairly suggests the invention positively recited in Claims 32-43. Moreover, there is no

teaching contained within the applied references themselves to suggest their being combined in the first instance.

The Toomik et al. reference has been addressed in the previous section, and those comment are incorporated herein. Briefly recapping, the method described by Toomik et al. is limited entirely to substrates specifically reactive with protein kinase A, protein kinase C, casein kinases I and II, and acid phosphatase. None of these enzymes are lipid or phospholipid kinases or phosphatases (enzymes that act on a lipid or phospholipid substrate). The peptide substrates of the protein kinases and acid phosphatases recited by Toomik et al. are not specifically reactive with lipid or phospholipid kinases or phosphatases. Lipids and phospholipids do not contain amino acid residues and do not contain peptide bonds. They are not substrates for the protein kinases described in the Toomik et al. reference. Insofar as all of Claims 32-43 positively require using a substrate that is specifically reactive with a lipid or phospholipid kinase or phosphatase, there is a fundamental and fatal gap in the teaching of the Toomik et al. reference as applied to these claims.

Combining Toomik et al. with both the Promega Technical Bulletin No. 547, and the Sartobind product literature does not cure the fundamental failing of the Toomik et al. reference because the **combined** documents still do not teach or suggest an assay that requires substrates that are specifically reactive with lipid and phospholipid kinases and phosphatases (a limitation positively recited in all of Claims 32-43).

Specifically, the Promega Bulletin describes SAM2-brand Biotin Capture Membranes. These membranes are specifically designed for capturing molecules labeled with biotin via the strong and specific affinity of biotin for streptavidin. In the exact same fashion as the primary reference to Toomik et al., the Promega Bulletin is limited entirely to a description of an assay method for **protein** kinases. This reference is wholly silent with regard to lipid and phospholipid kinases and phosphatases.

Additionally, the SAM2-brand membranes cannot be used in the experimental design described by Toomik et al. Toomik et al. uses the SPOTs-brand membranes,

which are pre-derivatized to contain reactive amino groups. These membranes, the only type disclosed in Toomik et al. are specifically designed for solid-phase peptide synthesis. In contrast, the SAM2-brand membranes are coated with avidin and designed to capture a biotin-tagged analyte from solution. If the teaching of Toomik et al. is modified to use Promega's SAM2-membrane (rather than the SPOTs-brand membrane), the utility of the Toomik et al. assay fails because a SAMs-membrane will not function in the solid-phase peptide chemistry described by Toomik et al.—unlike the SPOTs membrane, the SAM2 membrane lacks the immobilized, activatable amino groups onto which the solid-phase peptides are initiated.

The same conclusion is obtained when all three references are **combined**. The Sartobind-brand membrane literature is, by its explicit terms, wholly limited to applications where peptide and protein ligands are immobilized to the substrate. In fact, the introductory sentence of the Sartobind product brochure states unequivocally and without qualification that the "Sartobind aldehyde membranes [are] for coupling primary amine containing ligands **like proteins and peptides**." Emphasis added. So, while pre-formed peptides can be immobilized onto the Sartobind membrane, this membrane is not suitable as a substrate for solid-phase peptide synthesis—it lacks a derivatized anchor point that is present in the SPOTs membrane actually used by Toomik et al.

It is well-settled law that the Office cannot show a *prima facie* case of obviousness where the proposed combination of two or more references destroys the intended utility of the method described in the primary reference. See, for example, In re Gordon, 221 USPQ 1125 (Fed. Cir. 1984). In that instance, there is no technical motivation to make the proposed combination in the first place. In the present rejection, Toomik et al. clearly teach the need for a specialized substrate that can be used for solid-phase peptide synthesis. The Office proposed replacing that membrane with the membranes described in the Promega Literature or the Sartorious literature. But neither of these membranes are suitable for solid-phase peptide synthesis because they lack the activatable amino groups present in the SPOTs membrane described by

Toomik et al. Therefore, this rejection is improper because there is no technical motivation to combine the Toomik et al. reference with either of the Promega or Sartorious product literature.

Additionally, Applicants vehemently traverse the wholly unsupported statement in the Office Action that:

> One kinase or phosphatase is interchangeable for another because the reaction mechanisms of all kinases are the same and the reaction mechanisms for all phosphatases are the same. (Paper No. 12, page 7, 10-12.)

The Office provides no support whatsoever for this conclusion. Moreover, Applicants respectfully submit that this conclusion simply is not factually correct. Enzymes, even those of the same enzyme classification, vary vastly in their reaction mechanisms. Therefore they are not interchangeable at will, as the Office asserts.

By way of objective evidence of the differing nature of enzyme mechanics within kinases, Applicants submit for the Examiner's convenience Exhibits B, C, and D, which are representative abstracts of scientific papers that specifically address the mechanism of action of various kinases.

Exhibit B is an abstract of the 2001 lab work of the Louise N. Johnson lab at Oxford University. The abstract states that of the 27 or so protein kinase structures that have been solved by X-ray crystallography, while the enzymes share a common core structure, "each has a distinct substrate specificity and distinct mechanism for control." In short, these 27 extensively and exhaustively studied kinases operate by "disinct" mechanisms.

Exhibit C is an abstract of Schulze-Muth et al. (1996) J. Biol. Chem. 271:26684-89. This paper describes a receptor-like protein kinase from a plant, the Madagascar periwinkle. In comparing this protein kinase to other receptor-like protein kinases, the authors state that " In contrast to other plant receptor-like kinases, the kinase used an intra- rather than intermolecular phophorylation mechanism." In short, the protein kinase of from the Madagascar periwinkle operated by a different mechanism than previously known receptor-like protein kinases.

Exhibit D is the abstract of a grant effort headed by Louis Delbaere and supported by the Canadian government (Medical Research Council of Canada, Grant No. MT-10162, awarded to the principal author, Louis Delbaere). The abstract states that some kinases require only one divalent metal cation for optimal activity, while others require two divalent metal cations. Some transition states for these kinases are SN2-like or SN1-like. The different transition states have different geometries. This abstract indicates that various kinases operate by distinct mechanisms.

The documents presented in Exhibits B, C, and D document the fact that kinases and phosphatases do not all operate by the same mechanism, and are therefore not interchangeable, as suggested by the Office.

Thus, the Toomik et al., Promega, and Sartobind references are not combinable from the outset because the combination destroys the utility of the of the method described by Toomik et al. And when all three references are **combined**, the combination fails entirely to disclose or suggest an assay that requires the use of lipid and phospholipid kinases and phosphatases.

Applicants therefore submit that the rejection of Claims 32-43 under 35 U.S.C. 103(a) over Toomik et al. in view of Promega Technical Bulletin No. 547, and the Sartobind Product Literature is improper. Withdrawal of the same is respectfully requested.

Rejection of Claim 44 under 35 USC §103(a) over Toomik et al. in View of Shultz et al.:

This rejection is traversed because both the Toomik et al. and Shultz et al. references are limited to discussion of protein kinases. In contrast, Claim 44, which depends directly from Claim 31, is limited to a method that uses substrates for lipid and phospholipid kinases and phosphatases. These substrates are not, as asserted in the Office Action, peptides. By definition, a lipid or phospholipid kinase phosphorylates a lipid or phospholipid substrate. Likewise, a lipid or phospholipid phosphatase dephosphorylates a lipid or phospholipid substrate. Therefore, this rejection is

untenable because the teaching of the combined references is completely and wholly silent regarding an assay for any type of enzyme that acts upon a lipid or phospholipid substrate.

More specifically, the Office asserts that "one of skill in the art would have been motivated to modify Toomik et al. to use a crude lysate." But Toomik would use that crude lysate to look for a protein kinase, not a lipid or phospholipid kinase. Toomik would also need extensive knowledge of the substrate for the protein kinase because Toomik et al. require that the peptide substrate be synthesized *de novo*, using Fmoc-based techniques.

In short, the combination/modification forwarded by the Office does not result in the presently claimed invention, nor does it fairly suggest the presently claimed invention. When the **combination** is made, the method of Toomik et al. is used to assay a crude lysate as described by Shultz **for protein kinases**. The substrate for that protein kinase is not "contacted" with a binding matrix, as required by the base claim (Claim 31). Rather, the peptide substrate is synthesized from scratch on a specialized membrane, as explicitly disclosed by Toomik. Moreover, the peptide substrate itself, as taught by Toomik et al., is not "specifically reactive" with a lipid or phospholipid kinases or phosphatases, another positive limitation of the base claim. The Toomik et al. substrates are specifically reactive with protein kinases, a distinct class of substrates from those required by Claim 44.

Also, the Shultz reference does not utilize a crude cell extract in the fashion described by the Office. Specifically, in the Shultz patent, Example 7, column 22, lines 23-47, a pre-synthesized protein kinase substrate **was added directly** to a crude rat brain extract to see if the extract contained protein kinase C. In contrast, the Office states that "the crude extract was reacted with the kinase and the activity of the enzyme was detectable." That is actually not the case; the crude extract was not reacted with additional enzyme. As noted above: 1) the enzyme detected by Shultz was a protein kinase, not a lipid or phospholipid kinase; and 2) the enzyme itself was present in the crude extract from the outset and then substrate, fully formed, was added to the lysate.

Applicants therefore submit that the rejection of Claim 44 in view of Toomik et al. in view of Shultz is improper. Withdrawal of the same is respectfully requested.

Rejection of Claims 45 and 46 Under 35 USC §103(a) Over Toomik et al. in View of Alexis Biochemicals Product Literature, and Promega Technical Bulletin No. 547:

This rejection is respectfully traversed because 1) there is absolutely no suggestion or teaching in the combined references that the compound described in the Alexis Biochemicals product literature can be immobilized on the SPOTs membrane taught by Toomik or the Sartobind-brand membrane; and 2) even if it is immobilized, it is unknown and unpredictable whether the substrate would be available for reaction with a phospholipid kinase; and 3) the Toomik et al. reference and the Promega literature are limited entirely to protein kinases, a class of enzymes that will not react with the compound shown in the Alexis literature. Therefore, using the Alexis compound in either of the assays described in the Toomik et al. or Promega documents would totally destroy the utility of these two documents because they are limited entirely to testing for enzymes whose substrates are proteins, not lipids or phospholipids. Therefore, this rejection is improper.

The Alexis Product Data Sheet describes nothing more than the compound D-myo-inositol 4,5-bisphosphate, L-α-phosphatidyl- (1,2-dipalmitoyl) and states that the compound is soluble in DMSO.

It is not possible for one of skill in the art to extrapolate from the Alexis data sheet the conclusion that the Toomik et al. reference could be modified to apply an organic-phase solution of the Alexis compound to the matrix. In support of this proposed modification, the Office articulates two reasons: 1) the Alexis substrate dissolves easier; and 2) applying the Alexis substrate before the drying obviates a step. These two steps, however, are neither taught nor fairly suggested by the combined references. Thus the combined references themselves fail to suggest the modification proposed by the Office.

As described previously, Toomik et al. teaches only one type of substrate: a peptide substrate. And Toomik et al. teach only one way of immobilizing that substrate on a membrane: building the substrate from scratch via solid-phase peptide synthesis.

The Promega literature is likewise limited entirely to a teaching of one type of substrate: a peptide substrate. Moreover, the Promega SAM2-brand membrane is derivatized with streptavidin.

The interaction between the SPOTs membrane taught by Toomik et al. and the phospholipid taught by Alexis is wholly unknown based on the present record. Toomik provides absolutely no guidance on this matter because Toomik et al. teach only attaching peptides to the membrane using Fmoc-based peptide synthesis. Likewise, the Promega product is coated with streptavidin; a reaction (if any) between the Alexis phospholipid compound and the streptavidin is wholly unknown.

The **combined** references fail entirely to teach how to get the Alexis phospholipid adhered to a suitable substrate. The **combined** references fail entirely to teach that even if the Alexis phospholipid is immobilized, that it will be available for reaction with an enzyme in solution. The **combined** references fail entirely even to suggest combining the Alexis document with any of the other documents in the first instance because both the Promega literature and the Toomik et al. paper are concerned solely with protein kinases. The Alexis phospholipid compound is not a substrate for protein kinases. Thus, where is the motivation to combine the Alexis document with the others?

On this last point, Applicants note that the Office cannot use Applicants' own disclosure in hindsight to recreate the claimed invention. It is the applied references themselves, plus the body of knowledge that can be attributed to a person of ordinary skill in the art, that must be relied upon to provide the required motivation to combine two or more references. The Office Action explicitly states, in the sentence spanning the bottom of page 9 to the top of page 10, that it located the Alexis product literature from Applicants' own disclosure. The only motivation to combine the Alexis product

literature with the other applied references is supplied by Applicants' own disclosure. The Office is not at liberty to use Applicants' disclosure in this fashion.

If armed only with the Toomik et al. paper and the Promega literature, a person of ordinary skill in the art would not look at the Alexis literature and arrive at the present invention because the Toomik et al. paper and the Promega literature are entirely limited to discussion of enzymes that act upon peptide substrates. The Alexis compound is irrelevant to these types of enzymes because the compound is not a peptide. The only motivation to make this combination is provided by Applicants' own disclosure.

Applicants therefore submit that the rejection of Claims 45 and 46 under 35 USC §103(a) over Toomik et al. in view of the Alexis Biochemicals product literature and Promega Technical Bulletin No. 547 is improper. Withdrawal of the same is now requested.

CONCLUSION

Applicants submit that the objection and rejections have been traversed and that the application is ready for allowance. Early notification of the same is earnestly requested.

Respectfully submitted,

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. Serial No.: 09/871,424
Filing Date: 05/31/2001
Applicants: Goueli et al

Group Art Unit: 1651
Examiner: Guo, Lynda
Attorney Docket No.: 34506.105

Title: ASSAY FOR KINASES AND PHOSPHATASES

"MARKED UP" PARAGRAPHS AS AMENDED, 37 CFR §1.121(b)(1)(iii)

Fig. 10: Comparison of reaction products: the present invention vs. the conventional phospholipid extraction procedure. Activities were assessed using 1 μg of PI4P + 10 μg of PS as reaction substrates and purified PI-5K (2.5 ng) as the enzyme. The reaction was carried out for 10 minutes (lanes 1 and 2) and 0 minutes (lanes 3 and 4). The reaction was worked up using the conventional phospholipid extraction procedure as illustrated in **Fig. 1** (lanes 2 and 4) or according to the subject invention (lanes 1 and 3). When the reaction was **[been]** performed on membrane sheets, 40% of bound reaction products were re-extracted for TLC analysis.

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Title: ASSAY FOR KINASES AND PHOSPHATASES

"MARKED UP" CLAIMS AS AMENDED, 37 CFR §1.121(c)(1)(ii)

31. [AMENDED] A method for assaying presence, activity, or both, of an enzyme **[classified within an enzyme classification selected from the group consisting of EC 2.7.1, EC 3.1.3, and EC 3.1.4]**, the method comprising:

(a) contacting an enzyme substrate specifically reactive with an enzyme **[of a classification]** selected from the group consisting of **[EC 2.7.1, EC 3.1.3, and EC 3.1.4]** **a lipid kinase, a phospholipid kinase, a lipid phosphatase, and a phospholipid phosphatase** with a binding matrix; whereby the enzyme substrate is fixed to the matrix; and then

(b) contacting the substrate fixed to the matrix with an enzyme under conditions wherein the enzyme is active for a time sufficient to yield phosphorylated product fixed to the matrix when assaying a **lipid or phospholipid** kinase or a dephosphorylated product fixed to the matrix when assaying a **lipid or phospholipid** phosphatase; and then

(c) analyzing the matrix for presence of, amount of, or both the presence and the amount of the product fixed to the matrix, whereby the presence, the activity, or both the presence and activity of the enzyme can be determined.

34. [AMENDED] The method of Claim 32, wherein in step (a), the enzyme **substrate** is contacted with a binding matrix comprising an aldehyde-activated support.